

EFFECT OF 2,4-D AND PHENOLIC COMPOUNDS ON SEED GERMINATION AND GROWTH OF *PENNISETUM AMERICANUM* (L.) SCHUMANN AND THE LEACHING OF CHEMICALS IN SOIL

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Abstract

Relative toxicity of five phenolic compounds and 2,4-D herbicide on seed germination and early seedling growth of *Pennisetum americanum* (L.) Schumann and the degree of leaching of 2,4-D and benzoic acid in clay loam and sandy soil was studied. 2,4-D @ 10 and 50 ppm caused greater reduction in seed germination and root and shoot growth than the phenolic compounds. The phenolic compounds reduced seed germination percentage in the order: benzoic acid > p-coumaric > catechol > chlorogenic acid > gallic acid. A similar trend was observed with respect to root and shoot growth. 2,4-D in conjunction with p-coumaric acid and catechol exhibited synergistic effect on the process of germination and seedling growth. In the leaching experiment, greatest toxic effect was observed in the first 2 cm depth and lowest at 6 cm depth. Greater leaching of both 2,4-D and benzoic acid occurred in sandy soil compared to clay loam.

Introduction

The use of herbicides for weed control has developed rapidly since the mid-forties where phenoxyacetic herbicides such as MCPA (4-chloro-2-methyl-phenoxy acetic acid), 2,4-D (2,4-dichlorophenoxy acetic acid) and 2,4,5-T (2,4,5-trichlorophenoxy acetic acid) are widely used singly or in mixture as selective post-emergence herbicides against a variety of broad-leaf weeds (Fryer & Evans, 1977). An important characteristic of these chemicals is that they undergo rapid degradation in soil and are detoxified by microbial degradation, volatilization, photo-oxidation and irreversible adsorption on colloidal particles (Kearny & Kaufman, 1969; Anderson, 1977; Wilmington, 1985). Among the allelochemicals phenolic compounds are of common occurrence and accumulate in soils causing inhibition of both seed germination and overall growth of many plant species (Inderjit, 1998). Whitehead (1964) isolated p-coumaric and ferulic acids from four different soil types. Guenzi & McCalla (1966a,b) reported several phenolic compounds from different crop fields. Chou & Muller (1972) found considerable concentrations of p-coumaric and ferulic acids in soils under the shrub *Arctostaphylos glandulosa* var. *zacaensis* together with several other phytotoxins. Del Moral & Muller (1970) reported several phenolic compounds in the leachates from the litter of *Eucalyptus camaldelensis*. Usually a number of phenolic compounds present in the soil are collectively involved in allelopathic interactions (Blum, 1996). Lodhi (1975) observed cumulative effects of p-coumaric, ferulic and caffeic acid isolated from soils. A number of workers have reported inhibitory effects of phenolic acids on seed germination, shoot elongation and seedling growth (Olmsted & Rice, 1971; Kapustka & Rice, 1976; Rasmussen & Einhellig, 1977; Rice *et al.*, 1980; Williams &

Hoagland, 1982; Einhellig, 1987). The possibility of additive or synergistic effects of phenolic acids has been suggested (Wilson & Rice, 1968; Einhellig *et al.*, 1970; McCalla, 1971; Rasmussen & Einhellig, 1977; Dalton, 1989; Inderjit, 1996) and under field conditions the additive effects become more pronounced even at low concentrations compared to the effects of individual compounds (Einhellig, 1995).

Allelopathy is an important ecological process in natural and managed ecosystems (Rice, 1976). Both herbicides and phytotoxins that are added into the soil by allelopathic plants, are subjected to leaching by rain and irrigation water. Thus one method of detoxification of upper soil layer is leaching. However, the extent of leaching depends upon soil type.

The present report describes the relative toxicity of 5 phenolic compounds alone and in combination with 2,4-D herbicide and the extent of leaching of benzoic acid and 2,4-D in sandy and clay loam soils.

Materials and Methods

The phytotoxicity and leaching of 2,4-dichlorophenoxy acetic acid herbicide and five phenolic compounds viz., benzoic acid, gallic acid, p-coumaric acid, chlorogenic acid and catechol was studied. Seeds of bulrush millet, *Pennisetum americanum* (L.) Schumann var. White Hogari obtained from Pakistan Agricultural Research Council, Islamabad were used. Twenty seeds were placed in 9cm diam., sterilized Petri dishes containing Whatman No.1 filter paper soaked with 5 ml of 5, 10 and 50 ppm aqueous concentration of the chemicals. Distilled water was used as control. There were 5 replicates of each treatment. The Petri dishes were kept in a growth chamber maintained at 30°C day/25°C night (14 hours day length). Light intensity at the top of the dishes was 3000 Lux.

Germination of seeds were recorded daily upto 7 days. Emergence of radicle was taken as the criterion of seed germination. 2,4-D and phenolic acid mixture (1:1, w/w) in 100 and 200 ppm were also prepared to study the combined effect of herbicide and phenolic compounds viz., p-coumaric acid and catechol. Root and shoot lengths of the seedlings were measured at the end of the experiment.

To study leaching of the herbicide and benzoic acid, sandy and clay loam soils were used. The soils were air-dried, passed through 2 mm sieve and transferred in 8.0 cm diameter and 25 cm length plastic tubes in which 150 ml of 100 ppm and 200 ppm of 2,4-D or benzoic acid was poured at the top of each tube. In 2,4-D and benzoic acid combination, the final concentration contained the two chemicals in 1:1 ratio. The solution was allowed to leach in the tube for 3 days. Subsequently, soil sections from 2, 4 and 6 cm were obtained by cutting the tubes at respective lengths. The different soil portions were placed in earthen pots and 15 seeds of *P. americanum* were sown after imbibing for 3 hours. Similar experiment was carried out with the 1:1 mixture of benzoic acid and 2,4-D with the above mentioned concentrations and soil depths. Each treatment was replicated five times. Root and shoot lengths were measured after 8 days.

Data were subjected to factorial analysis of variance (FANOVA). Percentage data was transformed by an arcsin transformation (Zar, 1984).

Results

Germination: 2,4-D and five phenolic compounds were highly significant ($F=94.7, p<0.001$) while concentrations were also significant ($F=137.2, 159.4, p<0.01$). Compared to the phenolic compounds, 2,4-D caused both reduction in rate as well as the final germination percentage. The final germination percentage reduced progressively with the increase in concentration. Among the phenolic compounds benzoic acid was most inhibitory to germination (Fig.1). Final germination at 50 ppm concentration was reduced by the tested compounds in the order 2,4-D > benzoic acid > p-coumaric acid > catechol > chlorogenic acid > gallic acid.

In treatment where combinations of 2,4-D and the phenolic compounds (p-coumaric acid and catechol) was used greater reduction in the final germination percentage was observed compared to germination percentages when seeds were germinated in individual compounds (Fig.2). The synergistic effect was more pronounced in 2,4-D and p-coumaric acid combination where final germination percentage was significantly reduced even in 10 ppm concentration. At 50 ppm combined concentration there was a significantly greater reduction in germination percentage in 2,4-D and p-coumaric acid combination compared to that of 2,4-D and catechol ($p<0.01$).

Root and Shoot Growth: The root and shoot lengths of the seedlings were also reduced by 2,4-D and phenolic compounds (Fig.3). Herbicide 2,4-D greatly retarded the root and shoot growth as compared to the phenolic compounds at all concentrations. Among the phenolic compounds, greatest inhibition of root and shoot growth was produced by benzoic acid (Fig.3). Root growth at 50 ppm concentration of the tested compounds was inhibited in the order 2,4-D > benzoic acid > gallic acid > p-coumaric acid > catechol > chlorogenic acid whereas shoot growth at the same concentration was suppressed in the order 2,4-D > benzoic acid > p-coumaric acid > gallic acid > catechol > chlorogenic acid. As in germination, 2,4-D in conjunction with p-coumaric acid caused greater reduction of root and shoot growth compared to combination of 2,4-D and catechol (Fig.4).

Leaching experiment: In both the soil types combined effect of 2,4-D and benzoic acid on root and shoot growth was more pronounced in the upper 2 cm compared to 4 and 6 cm soil depth sections (Table 1). There was significantly greater leaching of the compounds in the sandy soil compared to clay-loam ($p<0.001$), as evidenced by consistently marked reduction in root and shoot growth at depths of 4 and 6 cm, compared to the clay loam soil at the corresponding depths. 2,4-D alone exhibited some degree of persistence in the upper 2 cm depth causing marked inhibition of root and shoot growth of *P. americanum*, particularly in the clay-loam soil. Likewise benzoic acid showed some retention in the top 2 cm as it caused marked reduction of root and shoot growth compared to 4 and 6 cm depth.

Discussion

Both 2,4-D herbicide and phenolic compounds showed inhibitory effects on seed germination and early seedling growth. The inhibitory effect of 2,4-D herbicide has

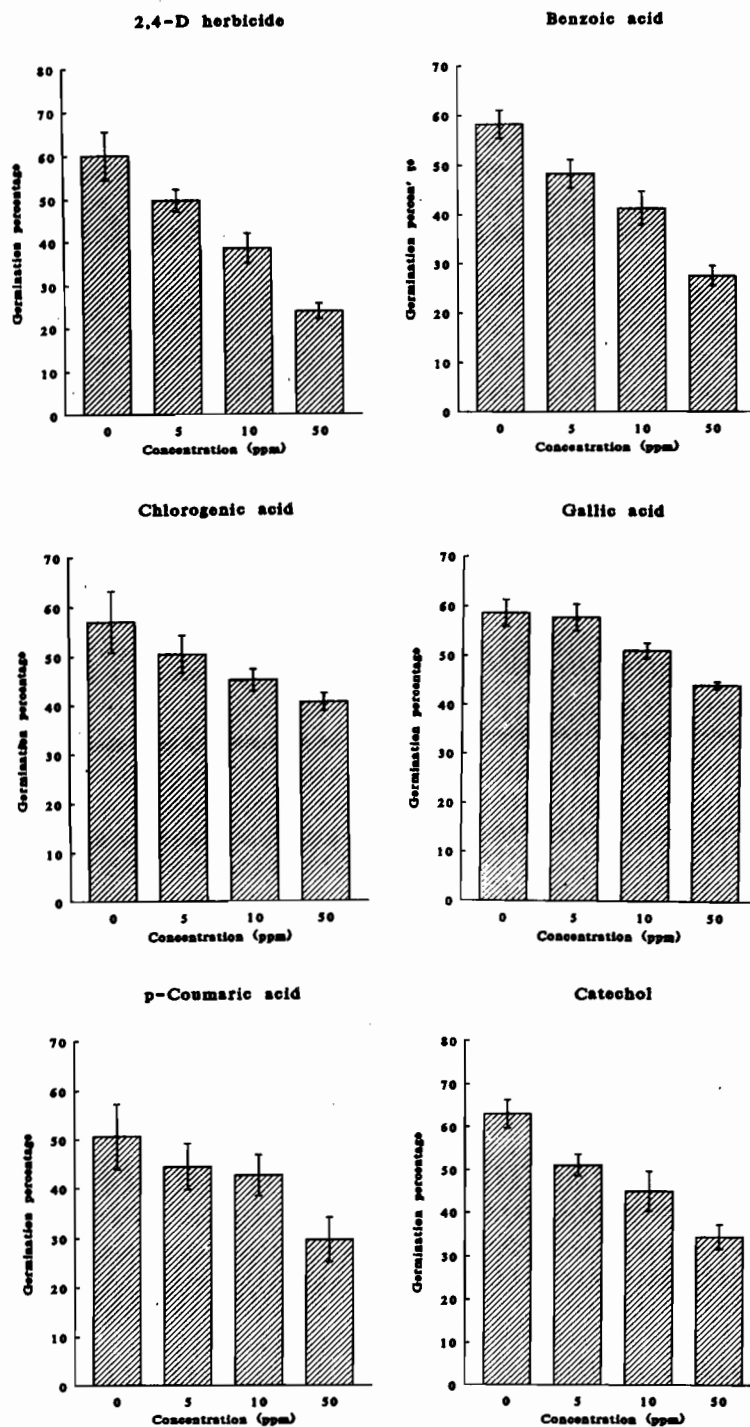


Fig.1. Effect of 2,4-D and five phenolic compounds on germination of *Pennisetum americanum*.

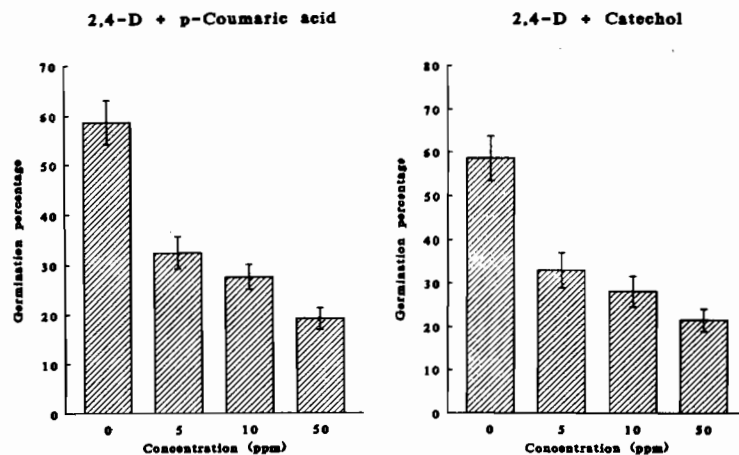


Fig.2. Effect of 2,4-D in conjunction with p-coumaric acid and catechol on germination of *Pennisetum americanum* (a) 2,4-D + p-coumaric acid, (b) 2,4-D + catechol.

been reported by Kozłowski & Sasaki, (1968); Shaukat & Soni (1974) Shaukat (1976) and Shaukat *et al.*, (1980). Reduction of germination by 2,4-D is the result of drastic inhibition of radicle (root) growth due to its strong phytohormonal action (Ashton, 1973; Audus, 1977). At high concentrations, 2,4-D also inhibits amylase activity (Shaukat, 1976). As the growing root and shoot, during early seedling development, require hydrolysed sugars, the inhibition of amylase activity also explains the suppression of root and shoot growth by 2,4-D. All the tested phenolic compounds exhibited inhibition of germination and seedling growth. Stowe *et al.*, (1987) also observed inhibition of growth and germination of tested species by p-coumaric acid and p-hydroxy benzoic acid. Rasmussen & Einhellig (1977, 1979) reported phytotoxic effect of p-coumaric acid and ferulic acid on the germination and growth of *Sorghum*. Perez (1990) found allelopathic effects of hydroxamic acid from cereals on *Avena sativa* and *A. fatua*. The water soluble phenolic compounds are added to the soil as they are leached from plant parts of allelopathic species or are returned to the soil in the decomposition process of such plants (Guenzi & McCalla, 1966a,b; Datta & Sinha-Roy, 1975; Shaukat *et al.*, 1983). Apparently a variety of plant processes may be modified by these phenolic phytotoxins. Several phenolic compounds including coumarin alter respiration rates in seeds and other plant organs (Van Sumere *et al.*, 1971; Koeppe, 1972; Lodhi & Nickell, 1973; Demos *et al.*, 1975) lowering tissue water content (Lodhi & Nickel, 1973), and inhibition of active ion transport through increased membrane permeability (Glass, 1974; Glass & Dunlap, 1974).

In the present study combined effect of phenolic compounds and 2,4-D showed synergistic action of the compounds. 2,4-D with p-coumaric acid was found to be more phytotoxic than that of catechol in combination with 2,4-D. 2,4-D with 200 ppm of coumarin exhibited a marked synergistic effect on germination and produced more severe toxicity symptoms compared to unadulterated 2,4-D. It suggests that the

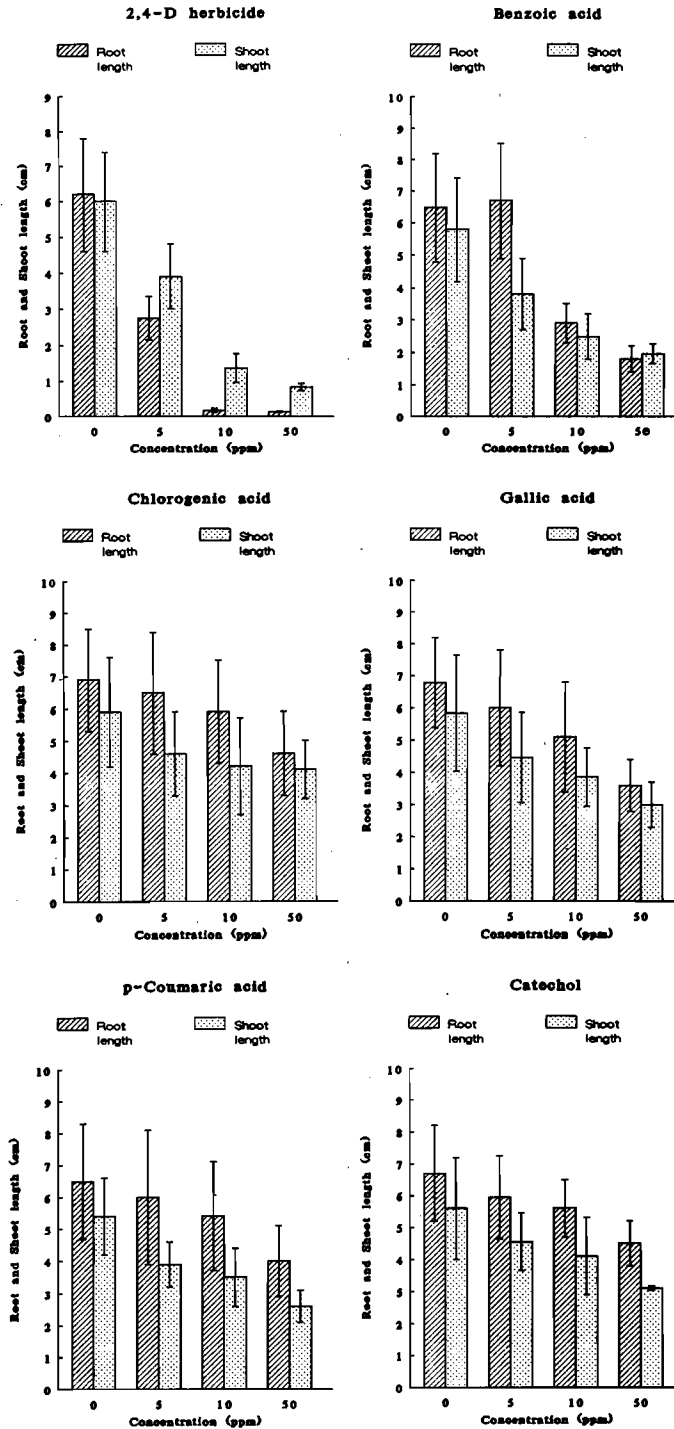


Fig.3. Effect of 2,4-D and five phenolic compounds on seedling growth of *Pennisetum americanum*.

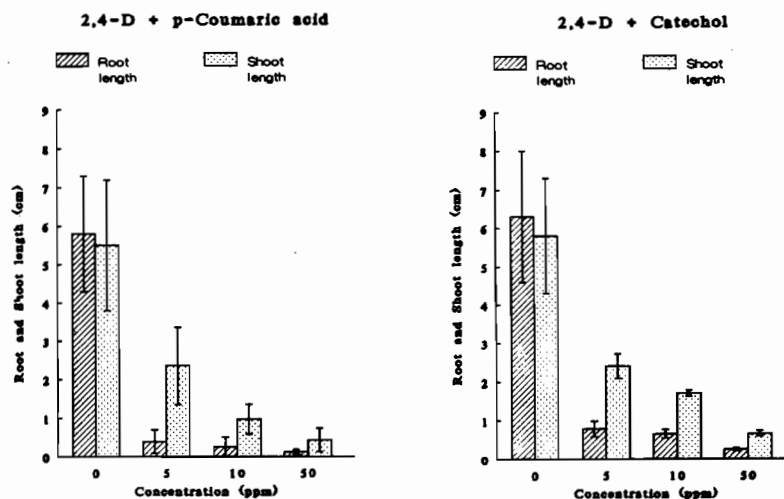


Fig.4. Effect of 2,4-D in conjunction with p-coumaric acid and catechol on seedling growth of *Pennisetum americanum*. (a) 2,4-D + p-coumaric acid, (b) 2,4-D + catechol.

programmes of herbicide application in soil should also take into account the amount and nature of phenolic compounds persisting in the soil because of possible synergistic action. Furthermore, herbicide concentration may correspondingly be reduced which may substantially curtail the cost of the chemical weed control.

Inhibition of root and shoot growth largely occurred in the pots containing the soil from upper 2 cm depth, showing insufficient leaching of the tested compounds to the deeper layers of soil. The leaching of phenolic compounds and herbicide in the soil may depend upon several factors such as the solubility of compounds in water, extent of irrigation, soil texture and organic matter percentage (Klingman, 1973). The herbicide molecules may also be adsorbed by the soil colloidal material which resists the leaching to deeper layers (Kearney & Kaufman, 1969; Wilmington, 1985).

Soil properties also have marked influence on herbicide availability and subsequent phytotoxicity (Shipman & Poonty, 1988). Greater leaching in the sandy soil than in clay loam soil resulted in a greater reduction in root and shoot growth at 4 and 6 cm depths compared to 2 cm depth. Similar results were obtained by Schiavau & Vandouresles (1988), who reported that in the leaching experiments the quantities of compounds leached depend not only upon applied dose, but also upon the possibilities of adsorption which were closely related to soil properties, in particular soil texture.

In the leaching experiment, 2,4-D and benzoic acid in combination significantly reduced root and shoot growth of bulrush millet over that of pure 2,4-D or benzoic acid, suggesting a synergistic action. Moreover, greater phytotoxic activity in the upper 2 cm layer in combination compared to separate application of these chemicals suggests lesser degree of leaching in combination. Einhellig (1987) reported that combination of atrazine and ferulic acid was much more inhibitory than the ferulic acid alone. Combinations of the herbicide trifluralin with ferulic acid or salicylic acid showed additive effect (Einhellig & Leather, 1988). Similarly, interactions of phenolic acids with the

Table 1. Activity of 2,4-D and benzoic acid (BA) after leaching as measured by shoot and root growth of *Pennisetum americanum* at different soil depths.

Concentration (ppm)		Soil types					
		Clay-loam			Sandy		
		Depth (cm)			Depth (cm)		
		2	4	6	2	4	6
Control	RL	4.30±0.92	3.90±0.82	4.00±1.22	3.00±0.94	3.20±0.80	0.50±0.95
	SL	5.50±0.74	5.00±0.96	5.30±1.16	4.00±0.71	4.10±0.74	4.50±1.23
100 2,4-D	RL	0.75±0.12	0.80±0.37	1.50±0.68	0.20±0.15	1.20±0.61	1.80±0.47
	SL	1.40±0.32	1.80±0.81	2.10±0.32	0.60±0.28	2.50±0.82	2.30±0.62
200 2,4-D	RL	0.80±0.32	0.30±0.16	1.50±0.22	0.25±0.18	0.60±0.36	1.20±0.39
	SL	0.90±0.27	1.20±0.42	1.80±0.38	0.60±0.35	0.90±0.28	1.70±0.71
100 Benzoic acid	RL	1.80±0.32	3.20±0.21	4.10±1.23	2.00±0.20	0.90±0.54	3.00±0.52
	SL	2.20±0.86	4.10±0.83	4.60±0.90	1.90±0.56	2.60±0.77	3.80±0.94
200 Benzoic acid	RL	1.60±0.78	2.80±0.42	4.00±1.11	1.90±0.52	2.60±0.52	3.10±1.24
	SL	2.60±0.64	3.80±0.63	4.50±0.84	1.50±0.36	2.20±0.36	3.30±1.15
100 BA+2,4-D	RL	0.35±0.22	2.20±0.28	3.90±0.86	0.15±0.21	2.80±0.21	3.00±0.83
	SL	1.40±0.08	3.00±0.42	5.00±0.71	0.90±0.33	3.10±0.36	3.20±1.04
200 BA+2,4-D	RL	0.14±0.07	1.50±0.38	3.50±0.51	0.05±0.04	1.00±0.20	2.80±0.98
	SL	0.50±0.24	2.60±0.81	4.20±1.33	0.21±0.08	1.70±0.31	2.50±1.12

SL = Shoot length, RL = root length (cm)

herbicides alachor and cinmetylin showed that in all cases, herbicide levels which alone had little impact on growth showed greater inhibitory effects in conjunction with phenolic acids (Wegher, 1986).

The success of any weed control treatment largely depends upon the presence of lethal concentration of a herbicide in the upper layers of soil, where most annual weed seeds germinate. An understanding of the relationship between leaching properties of a herbicide or an allelochemical and the root systems of weed/ crop plants is essential for successful weed control.

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(Received for publication 3 August 1998)